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Research Article

The Cognitive Ageing, Nutrition and Neurogenesis (CANN) trial: Design and progress

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Abstract

Introduction: The Cognitive Ageing, Nutrition and Neurogenesis trial hypothesizes that a combined intervention with long-chain n-3 polyunsaturated fatty acids (n-3) and cocoa flavan-3-ols (FLAV) will mitigate the cognitive decline anticipated to naturally occur over 1 year in older adults.

Methods: In a double-blinded, placebo-controlled parallel design, 259 individuals with mild cognitive impairment or subjective memory impairment were randomized to a control or n-3 FLAV group (1.5 g docosahexaenoic acid + eicosapentaenoic acid and 500 mg n-3 FLAV daily) for 12 months. Cognition was measured at 0, 3, and 12 months. The primary end-point is hippocampus-sensitive cognitive function (e.g., number of false-positives on the Picture Recognition Task of the Cognitive Drug Research test battery). Secondary outcomes include additional cognitive measures, brain atrophy and blood flow (assessed by magnetic resonance imaging), vascular function, circulating biomarkers of cardiovascular and cognitive health, gut microflora, red blood cell fatty acid status, and urine flavan-3-ol metabolites.

Results: Screening began in 2015, with all baseline visits completed in March 2017. The intervention was finished in March 2018.

Discussion: Cognitive Ageing, Nutrition and Neurogenesis aims to identify an effective diet-based intervention to prevent or delay cognitive impairment in cognitively at-risk individuals, which could ultimately contribute to a reduced population burden of dementia.

Conflicts of Interest: The research was funded in part by Abbott Nutrition via a Center for Nutrition, Learning, and Memory (CNLM) grant to the University of Illinois, which in turn awarded a research grant to the authors through a competitive peer reviewed process. Study foods were provided free of charge by Abbott Nutrition (Columbus, OH, USA). In addition, A.C. received funding from the US Highbush Blueberry Council. A.-M.M received funding from Unilever Best Foods. A.P. received funding and consultancy fees from Biostime, Blackmores, DSM, LifeVantage, Novasel Australia, Enzo Nutraceuticals, and Swisse Wellness. A.S. received funding from Arla Foods, Bayer, Kemin Foods, Nutricia-Danone, and Verdure Sciences. D.W. was funded by Arla Foods and Bayer. B.S. and N.C. were funded by Abbott Nutrition, Center for Learning and Memory, University of Illinois. D.V. received funding from the Florida Department of Citrus and the Cranberry Institute. K.W. is a consultant for Bracket, who provided the Cognitive Drug Research test battery for this study. T.D., S.D., R.G., M.I., G.J., R.K., R.L., and J.P. have no conflicts of interest to declare.

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Keywords: Cognition; Dementia; Mild cognitive impairment; Subjective memory impairment; Eicosapentaenoic acid; Docosahexaenoic acid; Cocoa flavan-3-ols; Hippocampus; Magnetic resonance imaging

1. Background and rationale

Although there is evidence of a decrease in incidence in early old age, the number of dementia cases is set to approximately double every 20 years, increasing to 115 million by 2050 [1]. Lifestyle strategies to preserve or improve memory and cognition would provide significant health, social, and economic benefits. In the UK, delaying dementia onset by 2 or 5 years would result in 19% and 33% reductions in prevalence, respectively, by 2050 with a much lower prevalence of severe dementia [2]. Similarly, it has been estimated that a 2-year delay in onset would reduce the global incidence of dementia by 22% by 2047 [3], resulting in 25 million fewer cases [1,3–5]. Even greater benefits would be observed in "at-risk" and prodromal population subgroups, such as apolipoprotein E4 (*APOE4*) carriers, and those with existing mild cognitive impairment (MCI) or subjective memory impairment (SMI; self-diagnosed and defined as noticing a decline in memory over the previous 2–3 years) [4–7]. Those with MCI and SMI, although experiencing metabolic brain changes and some loss of function, are unlikely to have yet undergone the same degree of macroscopic neural cell death in brain regions implicated in the pathogenesis of dementias as those with a confirmed diagnosis [8,9], and thus may be more responsive to interventions and experience some reversal of cognitive decline [10].

To date, the reductionist approach to nutritional interventions has often focused on the impact of individual foods, food groups, or dietary components on cognitive function. The neural processes involved in cognitive decline are complex and multifactorial. Therefore, combination treatments with dietary compounds that target multiple physiological and molecular mechanisms are likely to have the most realistic chance of affecting cognition [11,12].

Substantial data from prospective cohort studies and animal models provide evidence for the individual neurophysiological effects of the fish-derived long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as flavan-3-ols (n-3 FLAV; plant bioactives found in cocoa, teas, and berries). In a prospective study with 3.9 years of follow-up, higher total n-3 PUFA and DHA intakes were associated with a lower relative risk of Alzheimer's disease (AD; relative risk, 0.3; 95% CI, 0.1–0.9, in the highest quintile of DHA intake) [13], with subsequent observations of associations between plasma EPA and DHA status and dementia risk [14,15] and cognitive decline [15,16]. In the Women's Health Initiative Memory Study, EPA and DHA status was associated with larger total brain and hippocampal volumes, which was confirmed in more recent investigations [17,18]. The limited number of large-scale interventions with LC n-3 PUFA, predominantly in those with incident dementia, has produced mixed findings [19,20] and highlights the need to take a combined intervention approach with a focus on preclinical individuals.

Flavonoids are plant bioactives, with considerable evidence supporting their neurocognitive benefits. In the Personnes Agées QUID cohort, which included 1640 adults free of dementia aged 65 years or older, higher total flavonoid intake was associated with better cognitive performance at baseline and a better performance trajectory over a 10-year period [21]. An association between higher cocoa flavan-3-ol (which represents the focus of the current *Cognitive Ageing, Nutrition and Neurogenesis* [CANN] trial) consumption and performance on various cognitive instruments has been repeatedly observed [22–24], with Sorond and colleagues [24] also reporting an association with neurovascular coupling, which refers to the close functional and spatial relationship between cerebral blood flow (CBF) and neuronal activity.

Rodent studies provide substantial mechanistic insight [25–28] into the pleiotropic impact of EPA + DHA and n-3 FLAV, and suggest that they likely confer additive and potentially synergistic benefits. Neuronal membranes are particularly enriched in DHA, with DHA supplementation increasing DHA availability for cell membrane synthesis in neurogenesis, neurite outgrowth, and synaptogenesis [26]. Furthermore, DHA decreases neuroinflammation, mediated by the 15-lipoxygenase DHA derivative, neuroprotectin D1 [29]. n-3 FLAV promote neuronal survival, spine density formation, synaptic plasticity, and the production of brain-derived neurotrophic factor [25,27]. They are also anti-inflammatory and reduce the production of amyloid beta (40–42) peptides [30]. Through the enhancement of antioxidant enzyme activities, n-3 FLAV are also likely to indirectly reduce the oxidation of membrane DHA [31]. Finally, EPA + DHA plus n-3 FLAV may confer cognitive benefits through their impact on vascular function and CBF [32–35].

The CANN trial is designed to examine for the first time the impact of LC n-3 PUFA plus n-3 FLAV on cognition, brain atrophy and blood flow, and biomarkers of cognitive and cardiovascular function, over a 1-year period in older

adults with SMI or MCI. It aims to identify an intervention to promote cognitive health and reduce dementia risk, which would contribute to a reduced overall population burden of disease.

2. Design and methodology

2.1. Overview

This study uses a double-blinded, placebo-controlled parallel design. There are two recruitment sites: the University of East Anglia (Norwich, UK) and the Swinburne University of Technology (Melbourne, Australia), with magnetic resonance imaging (MRI) images analyzed centrally at the University of Illinois (Urbana Champaign, Urbana, IL). After a telephone screening, postal questionnaires, and an on-site screening visit (V1), participants are asked to attend three clinical visits (V2, V3, and V4) at 0 (baseline), 3, and 12 months (Fig. 1). At these visits, participants undergo cognitive assessment and provide a number of clinical measures and biological samples.

2.2. Ethical conduct of the study

The conduct, evaluation, and documentation of this study abide by Good Clinical Practice guidelines and the guiding principles of the Declaration of Helsinki. The study was approved by Bellberry Human Research Ethics Committee (Study ID 2015-03-227) and Swinburne University Human Research Ethics Committee (SHR Project 2015-208) for the Swinburne University of Technology site and the National Research Ethics Service Committee (Study ID 14/EE/0189) for the University of East Anglia site. All participants provided informed signed consent before participating.

2.3. Intervention products

Participants are asked to consume 3 × 1 g oil capsules (Captek Soft Gel International, Cerritos, CA) and 33 g of chocolate drops of either test or control products daily for 12 months, and to take the products with the main meal of the day (as there is some evidence of reduced EPA and DHA bioavailability in the fasting state relative to a fat-containing meal [36]). The test capsules provided fish oil (EPAX AS, Aalesund, Norway) delivering 1.1 g DHA and 0.4 g EPA per day. The control capsules contained a blend of 80% palm oil and 20% corn oil (Cargill and Hybco, Los Angeles, CA) that provides a fatty acid (FA) composition typical of a UK or Australian diet. Both the test and control capsules contained 1% lemon oil, to provide a lemon flavor to maintain study blinding, and 1% mixed tocopherols for stability of the study oils. The test chocolate drops (33 g) contained cocoa powder (Acticoa; Barry Callebaut, Lebbeke, Belgium) providing 500 mg of n-3 FLAV, ranging from monomers to decamers

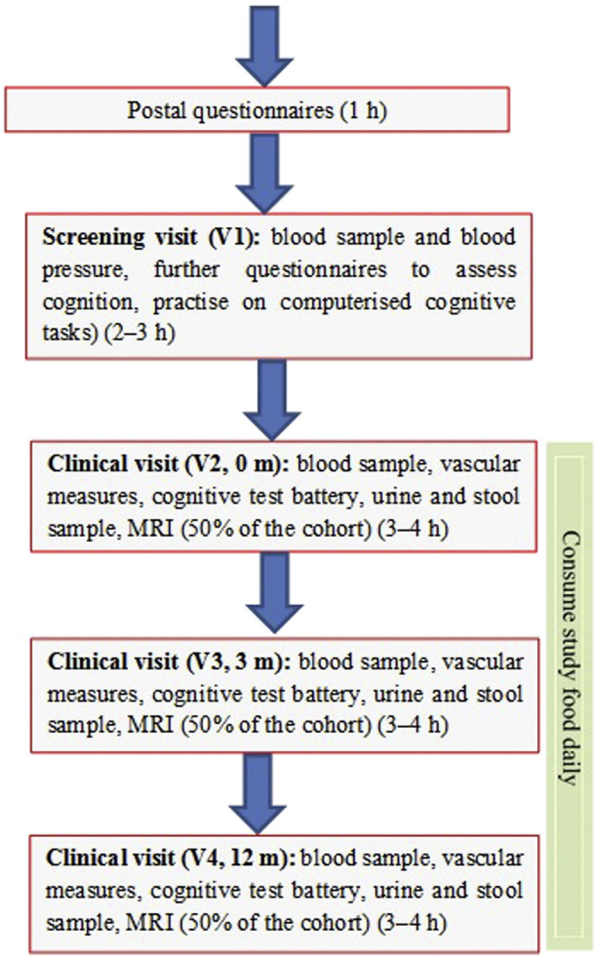

Fig. 1. Flowchart of study visits (~14 months). Abbreviation: MRI, magnetic resonance imaging.

(i.e., degrees of polymerization from 1 to 10). The test chocolate drops delivered 158 calories, 262 mg theobromine, and 30 mg caffeine. The control chocolate drops (33 g; Blommer Chocolate Co, Chicago, IL) were similar in size and color and delivered 38 mg n-3 FLAV, 160 calories, 64 mg theobromine, and 5.4 mg caffeine. The chocolates were matched for macronutrient composition. The doses of n-3 FAs and n-3 FLAV have been selected to be (1) physiologically relevant, equivalent to half a portion of oily fish and two portions of enriched cocoa per day, and (2) bioactive based on prospective epidemiologic data, as described previously.

At V2, participants are provided with their intervention products for the next 3 months, and at V3 participants receive the remaining 9 months' worth of supply. They are provided with a log to record the time at which the intervention products are consumed each day and are asked to return their empty chocolate sachets and oil capsule bottles to the research team to monitor compliance. The threshold for categorization as adherent to the intervention is set at 80%. Participants are contacted on a monthly basis

by telephone to promote compliance and to check for any adverse events or changes in health status, including medication use.

2.4. Study hypotheses

2.4.1. Primary hypothesis

Participants allocated to the intervention arm will perform significantly better than the control group on hippocampus-sensitive measures of cognitive function, namely the number of false-positives on the Picture Recognition Task of the Cognitive Drug Research (CDR) test battery and performance on the iPosition task.

2.4.2. Secondary hypotheses

1. Participants allocated to the intervention arm will perform better in the composite cognitive domains of attention, working memory, episodic memory, and speed of retrieval from memory, relative to the control intervention.
2. Participants allocated to the intervention arm and carrying the *APOE4* allele will show a better cognitive response to intervention relative to *APOE4* noncarriers.
3. Cognitive response to intervention will be associated with speciation of the gut microbiota.
4. Cerebrovascular blood flow and hippocampal volume, as assessed by MRI, will be greater in the intervention group compared with the control group at 12 months.

2.5. Participant inclusion criteria

All inclusion and exclusion criteria are detailed in Tables 1 and 2, respectively.

2.6. Sample size

The sample size estimate was based on the predicted impact of 1.5 g LC n-3 PUFA (1.1 g DHA and 0.4 g EPA) and 500 mg cocoa n-3 FLAV administered daily for 12 months. This combination has not been previously used in any intervention study and therefore a power calculation using directly relevant data was not possible. For LC n-3 PUFA, the most relevant previous study [37] administered 1.3 g DHA and 450 mg EPA for 12 months to MCI participants. Effects sizes, estimated from partial ηs, were as follows: for a delayed recall task, $\eta p^2 = 0.121$ (approx. $d = 0.74$); for the Digit Span task, $\eta p^2 = 0.254$ (approx. $d = 1.17$); and for a visual reproduction task, $\eta p^2 = 0.114$ (approx. $d = 0.72$). The computed d for a composite memory score (presented as a z-score) was 1.17. Regarding flavonoids, Desideri and colleagues administered 990 or 520 mg of n-3 FLAV, with a Cohen's d of 1.64 reported for the lower dose, which closely corresponds to that in our trial [22]. The outcome in this study was a composite cognitive score. The consumption of concord grape juice by MCI patients was associated with Cohen's f of 0.28 for verbal learning, and 0.33 for delayed recall (i.e., medium effect sizes) [38,39].

On the basis of the range of these effect sizes, we conservatively base our power calculation on a medium effect size, that is, Cohen's d of approximately 0.5, which generates a sample size of 108 per group for a two-arm trial, with 90% power to detect a significant change at the 5% probability level. The group sizes of 120 assumed a 10% attrition rate.

2.7. Study measures

Table 3 lists all the study procedures by visit.

2.8. Screening

The screening process was conducted in two phases.

2.8.1. Telephone screening

The CANN Telephone Screening Questionnaire assesses general adherence to the inclusion and exclusion criteria and provides an assessment of flavonoid, oily fish, and fish oil supplement intake. It also includes the Geriatric Depression Scale–short form (GDS-15) [40], the Modified Telephone

Table 1
Overview of CANN inclusion criteria

Type	Criterion
General	Males and females, aged 55 y and older
	Diagnosis of MCI or SMI with no indication of clinical dementia or depression
	Willing and able to provide written informed consent, and verbal informed consent for the telephone screening eligibility, and to comply with all study procedures
	Fluent in written and spoken English
	In good general health, including blood biochemical, hematologic, and urinalysis results within the normal range at screening (V1)
	Normal or corrected-to-normal vision and hearing
	Stable use of any prescribed medication for at least 4 wk before baseline (V2)
MRI (50% of cohort)	Aged from 55 to 85 y

Abbreviations: CANN, Cognitive Ageing, Nutrition and Neurogenesis; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; SMI, subjective memory impairment.

Table 2
Overview of CANN exclusion criteria

Type	Criterion
General and health-related	Diagnosis of AD or other form of dementia, or significant neurologic disorder
	Parent or sibling who developed dementia before the age of 60 y
	Past history/MRI evidence of brain damage, including significant trauma, stroke, or other serious neurologic disorder, including loss of consciousness for >24 h
	Clinically diagnosed psychiatric disorder likely to affect the cognitive measures
	Existing gastrointestinal disorder likely to impact on the absorption of flavonoids and FAs (e.g., celiac disease, Crohn disease, irritable bowel syndrome)
	Major cardiovascular event (e.g., myocardial infarction or stroke) in the last 12 mo
	Carotid stents or severe stenosis
	Any other existing medical condition likely to affect the study measures (as judged by the trial's clinical advisor)
	Uncontrolled hypertension (SBP >160 mm Hg or DBP >100 mm Hg)
	BMI $\geq$40 kg/m^2
	Current or ex-smoker who stopped <6 mo ago
	Prescribed medications likely to influence the study measures (as judged by the clinical advisor)
	Participated in any other study involving an investigational product in the last 4 wk
Drugs, alcohol, smoking	History of alcohol or drug dependency within the last 2 y
	Taking antipsychotics, high-dose antidepressants (as judged by the clinical advisor), cholinesterase inhibitors, benzodiazepines, anticonvulsants, illicit drugs/other drugs that can influence psychometric test results. Statins and NSAIDs are not exclusionary but use must be stable for at $\geq$2 mo before baseline (V2)
	Subjective reporting of sedation from any prescribed medication, that is, night sedation (Z drugs)/pain relief medication
Dietary-related	Known allergy to fish or any other component of the intervention products
	Taking flavonoid-containing supplements
	Taking fish oils or other n-3 FA-rich supplements
	High n-3 FA status (determined by red blood cell n-3 FA index >6% [EPA + DHA] as a % of total FAs)
	High flavonoid intake (>15 portions of flavonoid-rich foods per day)
MRI (50% of cohort)	Presence of metal implants
	Claustrophobia

Abbreviations: AD, Alzheimer's disease; BMI, body mass index; CANN, Cognitive Ageing, Nutrition and Neurogenesis; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; MRI, magnetic resonance imaging; NSAIDs, nonsteroidal anti-inflammatory drugs; SBP, systolic blood pressure.

Interview for Cognitive Status [41], and questions that serve as a basis for distinguishing SMI from MCI (confirmed at the on-site screening visit [V1]) (Fig. 2). Participants who passed the telephone screening were invited to take part in an in-person screening (V1) within 3 months. The following questionnaires were mailed out for completion before V1: the Functional Activities Questionnaire, Memory Functioning Questionnaire, NEO Personality Inventory-Five Factor Inventory, Hospital Anxiety and Depression Scale, and the CANN Study General Health and Lifestyle Questionnaire (see Appendix A).

2.8.2. *On-site screening visit (V1)*

Written consent was obtained from all participants at V1 after adequate explanation of the aims, methods, objectives, and potential hazards of the study. Date of birth, gender, total years of education, highest level of education, medical and surgical history (including allergies or food intolerances), and use of any concomitant medications were recorded in the case report form. Measurements of height and weight were taken for the calculation of body mass index (BMI) (with a BMI $\geq$40 kg/m^2 being exclusionary). Blood pressure (BP) was taken three times and the average of the second and third readings used as the BP value. If the average exceeded either 160 systolic BP or 100 diastolic BP (mm Hg), the participant was deemed ineligible for trial entry, and advised to consult with their general practitioner. A fasted blood sample was collected for *APOE* genotyping, red blood cell (RBC) FA analysis, full blood count, and liver and kidney function test (see Appendix A). Individuals with an RBC DHA level of >6% of total FAs, or a high intake of oily fish or flavonoid-rich foods (see Appendix A) were precluded from participating, on the basis that they had a high habitual intake of DHA and flavonoids, which are unrepresentative of the normal population and are unlikely to be responsive to intervention. Further details are given in Appendix A.

2.8.3. *Assessment of cognitive status*

After a standard breakfast, participants completed a neuropsychological test battery to confirm MCI or SMI status. The following instruments were administered: the Montreal Cognitive Assessment (MoCA) [42], California Verbal Learning Test-II [43], Boston Naming Test [44], Figure Copy task [45], Digit Span task (Forward and Backward) [46], Trail Making Test (A and B) [47], Test of Premorbid Functioning [48], CDR test battery [49], and iPosition task [50,51] (see Appendix B).

Table 3
Overview of CANN study testing procedures and sample collections by time point

Study event	Screening (V1)	Baseline (V2)	3 mo (V3)	12 mo (V4)
CANN Telephone Screening Questionnaire	X			
Functional Activities Questionnaire	X			X
Memory Functioning Questionnaire	X			X
NEO Personality Inventory-Five Factor Inventory	X			
Hospital Anxiety and Depression Scale	X			
General Health and Lifestyle Questionnaire	X			
Informed Consent	X			
Adverse events	X	X	X	X
Montreal Cognitive Assessment	X	X	X	X
Test of Premorbid Functioning	X			
California Verbal Learning Test-II	X			
Logical Memory I and II	X			
Boston Naming Test	X			
Figure Copy task	X			
Digit Span Task—Forward and Backward	X			
Trail Making Test—A and B	X			
International Physical Activity Questionnaire		X	X	X
Profile of Mood States		X	X	X
Food Frequency Questionnaire		X	X	X
Cognitive Drug Research Test Battery	[Practice]	X	X	X
iPosition		X	X	X
Verbal Fluency Test		X		X
Vascular function*		X	X	X
Plasma Biomarkers†		X	X	X
PBMC collection		X	X	X
Magnetic resonance imaging‡		X		X
Magnetoencephalography§		X	X	X
Blood sample for genotyping	X			
Urine collection		X	X	X
Stool collection		X	X	X

Abbreviations: CANN, Cognitive Ageing, Nutrition and Neurogenesis; PBMC, blood peripheral blood mononuclear cell.

NOTE. Red blood cell fatty acid status will be obtained as a measure of response to intervention.

*Resting blood pressure, central pressure and wave reflection, carotid/cerebral pulse wave velocity (plus ambulatory 24 hour blood pressure at the University of East Anglia, Norwich, UK, only).

†Lipids, glucose, inflammatory markers, and brain-derived neurotrophic factor.

‡Diffusion tensor imaging, arterial spin labeling, magnetic resonance spectroscopy (in 50% of the trial participants).

§At the Swinburne University of Technology, Melbourne, Australia, only.

For classification of MCI status, criteria developed by the National Institute on Aging-Alzheimer's Association workgroup [52] are used (Fig. 2). If a respondent feels that (1) their memory has declined in the last 2 to 3 years, and this is of concern to them, a diagnosis of MCI is possible depending on evidence of (2) preservation of independence in functional abilities (Functional Activities Questionnaire score <6), (3) absence of dementia (MoCA score ≥ 18), and (4) depression (GDS-15 score <10). Finally, impairment in one or more cognitive domains that are greater than would be expected given a person's age and education is required for MCI status (≥ 1 standard deviation [SD] below the mean on any of the following neuropsychological tests: memory, California Verbal Learning Test-II or Logical Memory I or II of the Wechsler Memory Scale–Revised; language, Boston Naming Test; visuospatial function, the Figure Copy task of the Repeatable Battery for the Assessment of Neuropsychological Status; attention, the Digit Span task (Forward or Backward) of the Wechsler Adult Intelligence Scale, third edition; and executive function, the Trail Making Test [A or B] [see Appendix C]). Participants who satisfy aforementioned criteria (1) to (4), but do not score ≥ 1 SD below the mean on any neuropsychological test are classified as SMI.

All eligible participants at V1 are sent the EPIC Food Frequency Questionnaire, the International Physical Activity Questionnaire, and the Profile of Mood States (see Appendix D).

2.9. Randomization of participants to groups

In total, 259 participants were recruited (by means of general practitioner surgeries, general advertising, newspaper articles, and radio show participation) and randomized to arm A or arm B (Fig. 3) using a randomization algorithm (Covariate Adaptive Randomization software [53]), with groups stratified by *APOE* genotype (E4 carrier vs. non-E4

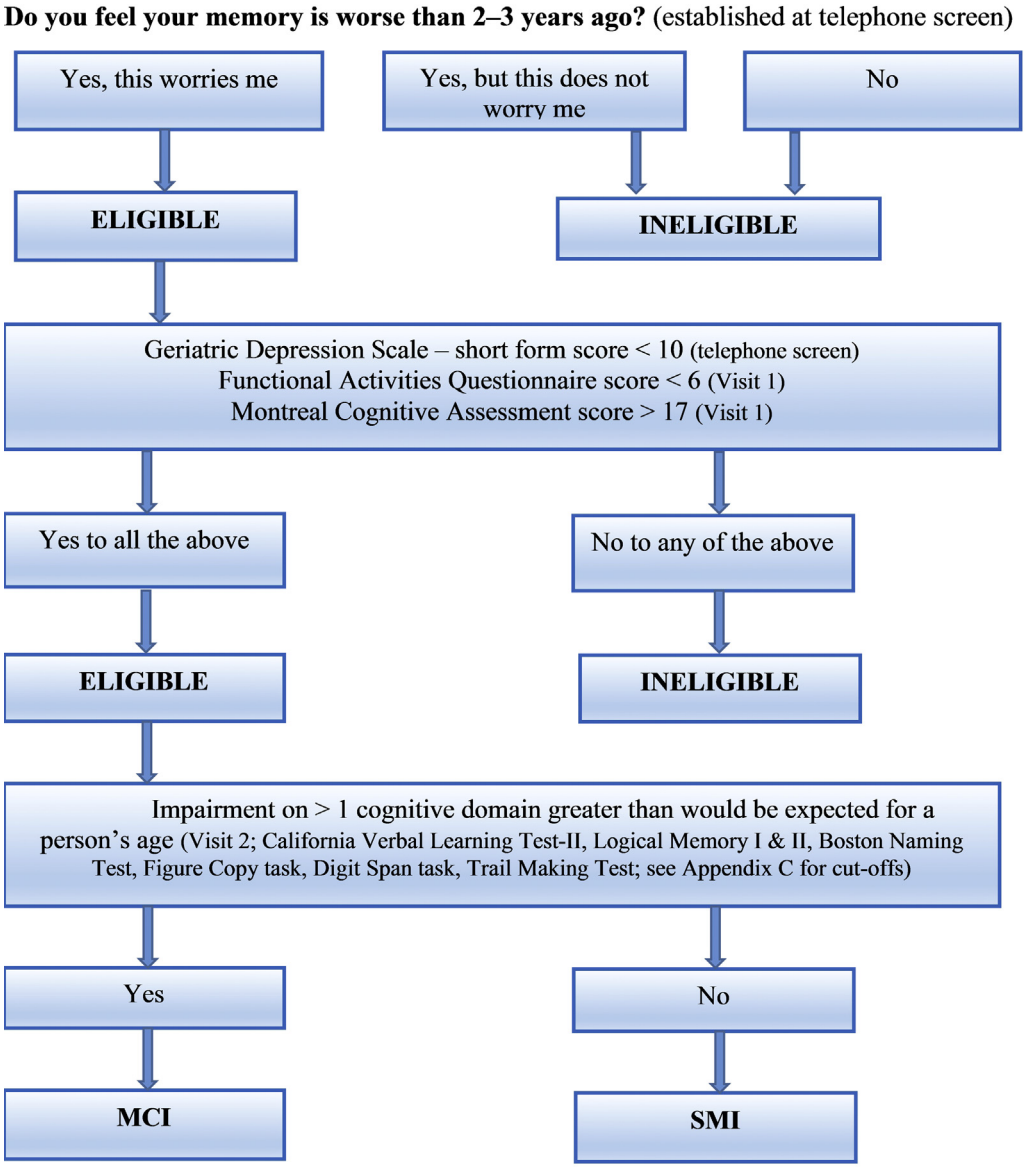

Fig. 2. Flowchart for classifying mild cognitive impairment (MCI) and subjective memory impairment (SMI) status.

carrier), sex (male vs. female), and cognitive status (SMI vs. MCI).

2.9.1. Clinical visits V2 to V4

The neuropsychological test battery for V2 to V4 comprises the CDR test battery, MoCA, Delis-Kaplan Executive Function System, Verbal Fluency Test (V2 and V4 only), and iPosition task (Appendix B). Participants provide an overnight fasted blood sample, a urine sample analyzed for flavan-3-ol metabolite profile, and a fecal sample to determine speciation and metabolism of the gut microbiota. Cardiovascular assessments, including aortic and 24-hour ambulatory BP and carotid-femoral pulse wave velocity were also conducted (for full details see Appendix E). The plasma samples will be analyzed for a number of biomarkers of cardiovascular and cognitive function including triglycerides, total and HDL cholesterol, glucose, insulin, cortisol, brain-derived neurotrophic factor, ApoE, and C-reactive protein (as a measure of inflammation).

2.9.2. MRI and magnetoencephalography (V2 and V4)

We use a variety of magnetic resonance techniques to characterize aspects of the brain that are sensitive to MCI. These include structural imaging, diffusion tensor imaging, magnetic resonance spectroscopy, arterial spin labeling, and magnetoencephalography. Diffusion tensor imaging provides a measure of white matter integrity that is sensitive

to MCI and AD [54]. Magnetic resonance spectroscopy is used to measure the biochemistry of the brain and has been used to obtain measures that are predictive of MCI [55]. In CANN, we will measure *N*-acetylaspartate, creatine, choline, glutamate, and myo-inositol as biomarkers of neurogenic activity. Arterial spin labeling has previously been used to monitor changes in CBF in patients with AD and MCI [56]. Protocol details for each imaging modality used in CANN are given in Appendix F.

3. Statistical analysis

Data will be analyzed using SAS (ver. 9.4; SAS Institute, Cary, NC) and SPSS software (ver. 22.0; SPSS Inc, Chicago, IL). Summary statistics (*n*, mean, median, SD, standard error of the mean, range) will be generated and change from baseline scores for treatment arms A and B, on all outcome measures, will be calculated by subtracting the baseline score (V2) from those at V3 and V4. Group comparisons will be performed using independent *t* tests and analysis of variance as appropriate, and correlation analyses will be conducted using Pearson's *r*.

4. Results

Fig. 3 shows the flow of participants throughout the study period. After the initial telephone screening (N = 637), prospective participants were invited to the on-site screen (V1, $n = 351$) within 3 months; those who were deemed eligible were then allocated to the intervention or control arm ($n = 259$) and invited back for their first clinical visit (V2) within 3 months. Two hundred forty-six participants completed V2, with a mean (±SD) age and BMI of 66.5 ± 6.5 years and 268.9 ± 4.3 kg/m^2, respectively. In total, 57% of the cohort was female and 28%

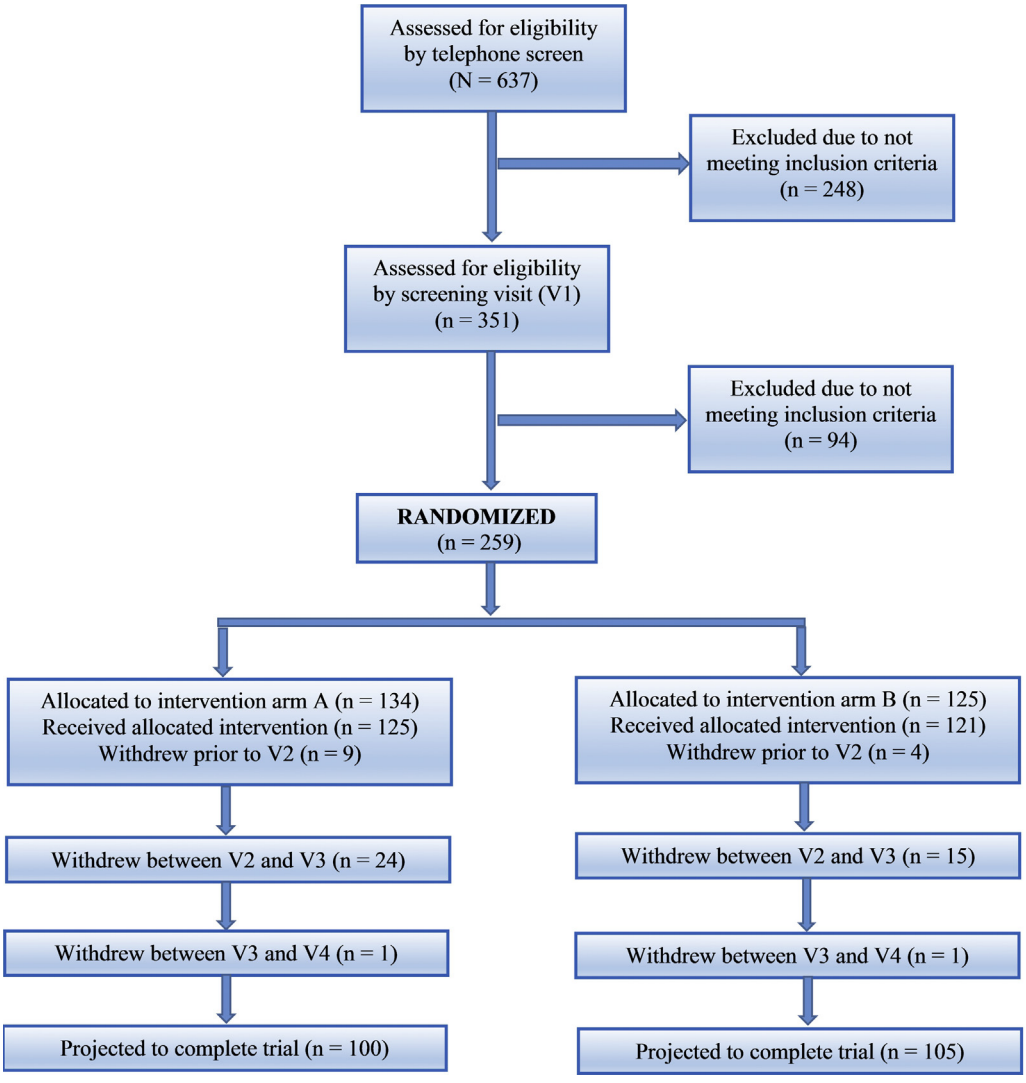

Fig. 3. Flowchart of participation in the Cognitive Ageing, Nutrition and Neurogenesis (CANN) study.

Table 4
Demographic characteristics of study participants

Characteristic	Screening visit (V1) (n = 351)	Baseline clinical visit (V2)	
		Arm A (n = 125)	Arm B (n = 121)
Age (y)	66.1 ± 6.5	65.7 ± 6.2	64.8 ± 6.6
Gender			
Male	148	55	51
Female	203	70	70
Cognitive status			
SMI	201	71	71
MCI	140	54	50
APOE4 carrier			
Yes	96	35	34
No	242	90	87
MoCA score	27.0 ± 2.4	27.0 ± 2.3	27.1 ± 2.3
Years of education	14.5 ± 3.8	14.9 ± 4.2	14.1 ± 3.2
IQ	115.6 ± 9.4	116.0 ± 9.8	115.3 ± 9.3
BMI	27.3 ± 6.1	27.1 ± 4.1	26.6 ± 4.4
RBC n-3 FA index	5.2 ± 1.3	4.7 ± 1.0	4.7 ± 0.8
SBP (mm Hg)	139.7 ± 15.3	138.1 ± 14.32	138.8 ± 14.9
DBP (mm Hg)	81.0 ± 9.0	81.8 ± 8.8	80.5 ± 8.5

Abbreviations: *APOE4*, apolipoprotein E4; BMI, body mass index; DBP, diastolic blood pressure; IQ, intelligence quotient; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; RBC n-3 FA index, red blood cell n-3 fatty acid index (=% of total fatty acid as eicosapentaenoic acid plus docosahexaenoic acid); SBP, systolic blood pressure; SMI, subjective memory impairment.

NOTE. Values are numbers or means ± standard deviation. There were no significant differences between the two treatment arms for any parameter (all $P > .05$). On analysis of study dropouts versus project completed, no significant differences were found in any variable listed in Table (all $P > .05$), with the exception of gender ($P = .003$), with the dropouts being significantly more likely to be female.

were *APOE4* carriers; there was an MCI/SMI ratio of 104:142 (Table 4).

5. Discussion

CANN will investigate if an intervention with a combination of dietary components can prevent or delay cognitive decline in older adults at increased risk of dementia. The choice of outcome measures has long been a subject of debate for randomized control trials targeting dementia risk. Given that the focus in CANN is on prevention rather than on conversion of SMI/MCI to AD, sensitive neurocognitive tests were used to test efficacy with false-positives in the Picture Recognition Task and the iPosition task chosen as highly sensitive indices of hippocampus integrity and function.

The inclusion of structural and functional MRI and magnetoencephalography, along with a range of prognostic cardiovascular assessments, such as 24-hour ambulatory BP and pulse wave velocity, will provide insight into the physiological basis of the impact of intervention on cognition. Cardiovascular health is being increasingly recognized as an important and modifiable determinant of neurocognitive function and dementia risk [57], influencing perfusion, blood-brain barrier function, and brain inflammatory and lipid status. Given the cardiovascular benefits of LC n-3 PUFA [58] and n-3 FLAV [59], improved cardiovascular function is likely to underlie any observed cognitive benefits.

A further design strength is the use of RBC FA status (n-3 index) as an exclusionary criterion. Individuals with a total RBC n-3 index of >6% were ineligible on the basis that they are unrepresentative of the general population. This subgroup constitutes the "health conscious" older adult deciles, with an EPA + DHA intake of about 300 mg per day provided by fish oil supplements or at least one portion of oily fish per week [60,61]; those with such a high baseline status are unlikely to be responsive to additional intake. Most previous trials have used fish oil supplements (rather than EPA + DHA status) as an exclusionary criterion, with high oily fish consumers often included, which may have contributed to the observed lack of responsiveness to intervention [62].

In the UK, about 60% of the population aged >55 years are on prescribed medications, which increases to over 90% of those aged 85 years and older [63]. The CANN trial took a pragmatic approach to maximize population applicability, with only those on high-dose medications directly targeting neurologic functions precluded from participating. For all other medications, participants were required to have stable use for at least 2 months before the baseline assessment.

APOE genotype is the strongest common genetic risk factor for cognitive decline and AD, with those with *APOE3/E4* and *APOE4/E4* genotypes at a 4- and 16-fold increased risk of AD relative to the wild-type *APOE3/E3* genotype [64]. There is accumulating evidence that part of the neurologic dysfunction in *APOE4* carriers is mediated by altered DHA metabolism, which in healthy and prodromal individuals could be mitigated by increased DHA intake and status. Although not fully powered to establish *APOE4* × treatment interactions, *APOE4* carrier status was used to randomize the participants to the intervention arm, and an exploratory analysis was conducted to assess response to intervention according to the genotype.

Given the ever-increasing population burden of dementias, the lack of effective therapeutics, and the large impact on population prevalence of even modest delays in disease onset, the outcomes of the CANN trial are of substantial public health relevance.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.trci.2018.08.001.

RESEARCH IN CONTEXT

1. Systematic review: Search of PubMed and Web of Science databases for articles on dietary interventions for cognitive decline and/or dementia, including the terms "cognition," "dementia," "Alzheimer's," "mild cognitive impairment," "subjective memory impairment," "omega-3 fatty acids," "n-3 fatty acids," "flavan-3-ols," and so forth. The literature suggests long-chain n-3 polyunsaturated fatty acid and flavonoids may improve cognition but there are important gaps regarding their possible synergistic effects.
2. Interpretation: Our trial is the first to investigate the impact of cosupplementation with marine n-3 fatty acid and cocoa flavan-3-ols on cognition and brain structure/function (by magnetic resonance imaging) in older adults at risk of dementia. Delaying onset by 2 to 5 years would reduce population burden in the UK by 19% to 33% by 2050, respectively.
3. Future directions: Future studies should explore the effects of the timing (within the healthy-to-advanced dementia disease trajectory) of intervention with flavan-3-ols in producing the maximum cognitive benefits and also to examine response to intervention according to *APOE* genotype status.

References

[1] Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement 2013;9:63–75.e2.

[2] Lewis F, Schaffer SK, Sussex J, O'Neill P, Cockcroft L. The trajectory of dementia in the UK—making a difference. London: Office of Health Economics; 2014.

[3] Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health 1998;88:1337–42.

[4] Jessen F, Wiese B, Bachmann C, Eifflaender-Gorfer S, Haller F, Kolsch H, et al. Prediction of dementia by subjective memory impairment: effects of severity and temporal association with cognitive impairment. Arch Gen Psychiatry 2010;67:414–22.

[5] Alzheimer's Disease International (ADI), World Alzheimer Report 2015. The Global Impact of Dementia. London: ADI; 2015.

[6] Jessen F, Wolfsgruber S, Wiese B, Bickel H, Mosch E, Kaduszkiewicz H, et al. AD dementia risk in late MCI, in early MCI, and in subjective memory impairment. Alzheimers Dement 2014;10:76–83.

[7] Wolfsgruber S, Wagner M, Schmidtke K, Frolich L, Kurz A, Schulz S, et al. Memory concerns, memory performance and risk of dementia in patients with mild cognitive impairment. PLoS One 2014;9:e100812.

[8] De Santi S, de Leon MJ, Rusinek H, Convit A, Tarshish CY, Roche A, et al. Hippocampal formation glucose metabolism and volume losses in MCI and AD. Neurobiol Aging 2001;22:529–39.

[9] Karas GB, Scheltens P, Rombouts SA, Visser PJ, van Schijndel RA, Fox NC, et al. Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. Neuroimage 2004;23:708–16.

[10] Canevelli M, Grande G, Lacorte E, Quarchioni E, Cesari M, Mariani C, et al. Spontaneous reversion of mild cognitive impairment to normal cognition: a systematic review of literature and meta-analysis. J Am Med Dir Assoc 2016;17:943–8.

[11] Frautschy SA, Cole GM. Why pleiotropic interventions are needed for Alzheimer's disease. Mol Neurobiol 2010;41:392–409.

[12] Grimm MO, Michaelson D, Hartmann T. Omega-3 fatty acids, lipids and apoE lipidation in Alzheimer's disease: a rationale for multi-nutrient dementia prevention. J Lipid Res 2017;58:2083–101.

[13] Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940–6.

[14] Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545–50.

[15] Beydoun MA, Feng Q, Azizkhanian I, Rawat V, Castor K, Fonteh AN, et al. Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. Am J Clin Nutr 2007;85:1103–11.

[16] Dullemeijer C, Durga J, Brouwer IA, van de Rest O, Kok FJ, Brummer RJ, et al. n 3 Fatty acid proportions in plasma and cognitive performance in older adults. Am J Clin Nutr 2007;86:1479–85.

[17] Pottala JV, Yaffe K, Robinson JG, Espeland MA, Wallace R, Harris WS. Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI study. Neurology 2014;82:435–42.

[18] Yassine HN, Feng Q, Azizkhanian I, Rawat V, Castor K, Fonteh AN, et al. Association of serum docosahexaenoic acid with cerebral amyloidosis. JAMA Neurol 2016;73:1208–16.

[19] Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C, et al. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. JAMA 2010; 304:1903–11.

[20] Stonehouse W, Conlon CA, Podd J, Hill SR, Minihane AM, Haskell C, et al. DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial. Am J Clin Nutr 2013;97:1134–43.

[21] Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P. Flavonoid intake and cognitive decline over a 10-year period. Am J Epidemiol 2007;165:1364–71.

[22] Desideri G, Kwik-Uribe C, Grassi D, Necozione S, Ghiadoni L, Mastroiacovo D, et al. Benefits in cognitive function, blood pressure, and insulin resistance through cocoa flavanol consumption in elderly subjects with mild cognitive impairment the Cocoa, Cognition, and Aging (CoCoA) study. Hypertension 2012;60:794–801.

[23] Neshatdoust S, Saunders C, Castle SM, Vauzour D, Williams C, Butler L, et al. High-flavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: two randomised, controlled trials. Nutr Healthy Aging 2016;4:81–93.

[24] Sorond FA, Hurwitz S, Salat DH, Greve DN, Fisher ND. Neurovascular coupling, cerebral white matter integrity, and response to cocoa in older people. Neurology 2013;81:904–9.

[25] Williams RJ, Spencer JP. Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med 2012;52:35–45.

[26] Oster T, Pillot T. Docosahexaenoic acid and synaptic protection in Alzheimer's disease mice. Biochim Biophys Acta 2010;1801:791–8.

[27] Williams CM, El Mohsen MA, Vauzour D, Rendeiro C, Butler LT, Ellis JA, et al. Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and

brain-derived neurotrophic factor (BDNF) levels. Free Radic Biol Med 2008;45:295–305.
[28] Boudrault C, Bazinet RP, Ma DW. Experimental models and mechanisms underlying the protective effects of n-3 polyunsaturated fatty acids in Alzheimer's disease. J Nutr Biochem 2009;20:1–10.
[29] Bazan NG. The docosanoid neuroprotectin D1 induces homeostatic regulation of neuroinflammation and cell survival. Prostaglandins Leukot Essent Fatty Acids 2013;88:127–9.
[30] Dal-Pan A, Julien C, Pierrisnard C, Tremblay C, Calon F. Resveratrol and, to a lesser extent, docosahexaenoic acid, induce disease-modifying effects in old 3xTg-AD mice. Alzheimer's Dement 2014; 8:182.
[31] Frautschy SA, Cole GM. What was lost in translation in the DHA trial is whom you should intend to treat. Alzheimers Res Ther 2011;3:2.
[32] Armah CK, Jackson KG, Doman I, James L, Cheghani F, Minihane AM. Fish oil fatty acids improve postprandial vascular reactivity in healthy men. Clin Sci (Lond) 2008;114:679–86.
[33] Kay CD, Hooper L, Kroon PA, Rimm EB, Cassidy A. Relative impact of flavonoid composition, dose and structure on vascular function: a systematic review of randomised controlled trials of flavonoid-rich food products. Mol Nutr Food Res 2012;56:1605–16.
[34] Jackson PA, Reay JL, Scholey AB, Kennedy DO. DHA-rich oil modulates the cerebral haemodynamic response to cognitive tasks in healthy young adults: a near IR spectroscopy pilot study. Br J Nutr 2012;107:1093–8.
[35] Jackson PA, Reay JL, Scholey AB, Kennedy DO. Docosahexaenoic acid-rich fish oil modulates the cerebral hemodynamic response to cognitive tasks in healthy young adults. Biol Psychol 2012;89:183–90.
[36] von Schacky C. Omega-3 fatty acids in cardiovascular disease—an uphill battle. Prostaglandins Leukot Essent Fatty Acids 2015;92:41–7.
[37] Lee LK, Shahar S, Rajab N, Yusoff NAM, Jamal RA, Then SM. The role of long chain omega-3 polyunsaturated fatty acids in reducing lipid peroxidation among elderly patients with mild cognitive impairment: a case-control study. J Nutr Biochem 2013;24:803–8.
[38] Krikorian R, Nash TA, Shidler MD, Shukitt-Hale B, Joseph JA. Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. Br J Nutr 2010;103:730–4.
[39] Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD, Shidler MD, et al. Concord grape juice supplementation and neurocognitive function in human aging. J Agric Food Chem 2012;60:5736–42.
[40] Sheikh JI, Yesavage JA. Geriatric Depression Scale (GDS): recent evidence and development of a shorter version. Clin Gerontologist 1986;5:165–73.
[41] Cook SE, Marsiske M, McCoy KJM. The use of the Modified Telephone Interview for Cognitive Status (TICS-M) in the detection of amnestic mild cognitive impairment. J Geriatr Psychiatry Neurol 2009;22:103–9.
[42] Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53:695–9.
[43] Delis D, Kramer J, Kaplan E, Ober B. California Verbal Learning Test. 2nd ed. San Antonio, TX: The Psychological Corporation; 2000.
[44] Kaplan E, Goodglass H, Weintraub S. Boston Naming Test. Philadelphia: Lea & Febriger; 1983.
[45] Randolph C, Tierney MC, Mohr E, Chase TN. The repeatable battery for the assessment of neuropsychological status (RBANS): preliminary clinical validity. J Clin Exp Neuropsychol 1998;20:310–9.
[46] Wechsler D. Wechsler Adult Intelligence Scale. 3rd ed. San Antonio, TX: The Psychological Corporation.; 1997.
[47] Reitan RM. The relation of the trail making test to organic brain damage. J Consult Psychol 1955;19:393–4.
[48] Wechsler D. Test of Premorbid Functioning. San Antonio, TX: The Psychological Corporation; 2009.
[49] Simpson PM, Surmon DJ, Wesnes KA, Wilcock GK. The cognitive drug research computerized assessment system for demented patients—a validation study. Int J Geriatr Psychiatry 1991;6:95–102.
[50] Watson PD, Voss JL, Warren DE, Tranel D, Cohen NJ. Spatial reconstruction by patients with hippocampal damage is dominated by relational memory errors. Hippocampus 2013;23:570–80.
[51] Nutter-Upham KE, Saykin AJ, Rabin LA, Roth RM, Wishart HA, Pare N, et al. Verbal fluency performance in amnestic MCI and older adults with cognitive complaints. Arch Clin Neuropsychol 2008; 23:229–41.
[52] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:270–9.
[53] Kang M, Ragan BG, Park JH. Issues in outcomes research: an overview of randomization techniques for clinical trials. J Athl Train 2008;43:215–21.
[54] Stebbins GT, Murphy CM. Diffusion tensor imaging in Alzheimer's disease and mild cognitive impairment. Behav Neurol 2009;21:39–49.
[55] Kantarci K, Weigand SD, Przybelski SA, Preboske GM, Pankratz VS, Vemuri P, et al. MRI and MRS predictors of mild cognitive impairment in a population-based sample. Neurology 2013;81:126–33.
[56] Alsop DC, Dai W, Grossman M, Detre JA. Arterial spin labeling blood flow MRI: its role in the early characterization of Alzheimer's disease. J Alzheimers Dis 2010;20:871–80.
[57] Qiu C, Fratiglioni L. A major role for cardiovascular burden in age-related cognitive decline. Nat Rev Cardiol 2015;12:267–77.
[58] Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 2002;287:1815–21.
[59] Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. Am J Clin Nutr 2012;95:740–51.
[60] Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized controlled trial. J Am Heart Assoc 2013;2:e000513.
[61] Minihane AM. Fish oil omega-3 fatty acids and cardio-metabolic health, alone or with statins. Eur J Clin Nutr 2013;67:536–40.
[62] Dangour AD, Allen E, Elbourne D, Fasey N, Fletcher AE, Hardy P, et al. Effect of 2-y n-3 long-chain polyunsaturated fatty acid supplementation on cognitive function in older people: a randomized, double-blind, controlled trial. Am J Clin Nutr 2010;91:1725–32.
[63] Chaplin S. Health Survey for England 2013: the use of prescribed medications. Prescriber; 2015. p. 16–9.
[64] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 2007;39:17–23.